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CAPTION FOR FRONT COVER:

Nuclear polyhedrosis virus-killed gypsy moth (Lymantria dispar (L.)) larvae on a tree trunk (left) and in a burlap trap (right).

AERIAL SPRAY TRIALS WITH DISPARVIRUS IN ONTARIO IN 1986

by

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ABSTRACT

Two plots infested with gypsy moth, *Lymantria dispar* (L.), were aerially sprayed with an aqueous formulation of Disparvirus, a nuclear polyhedrosis virus (NPV), containing 25% molasses and 60 g/L Orzan LS, to determine its efficacy. Plot 1 received a double application of 2.2×10^{12} polyhedral inclusion bodies (PIBs)/ha and plot 2 received a double application of 2.7×10^{11} PIBs/ha. Applications at 9.4 L/ha were 5 days apart when insects were in the first and second instars.

Excellent spray coverage was achieved and virus epizootics occurred in both plots with the highest levels of larval infection at 46.7% and 66.5% for plots 1 and 2 respectively. Highest infection levels of naturally occurring NPV recorded in check plot 1 and check plot 2 were 17.6% and 27.9% respectively. In treated plots, egg mass density fell from a pre-spray count of 1540/ha to a post-spray count of 10/ha in plot 1 and from 3240/ha to 1560/ha in plot 2. Populations in check plots also declined dramatically. Results from these trials indicate that Disparvirus is potentially effective as a control agent for gypsy moth.

RÉSUMÉ

Deux parcelles infestées par la spongieuse (*Lymantria dispar* [L.]) ont été soumises à des pulvérisations aériennes d'une formulation aqueuse de Disparvirus, un virus de la polyédrose nucléaire, contenant 25% de mélasse et 60 g/L d'Orzan LS, afin de déterminer son efficacité. La parcelle 1 a reçu une double application de $2,2 \times 10^{12}$ de corps d'inclusion polyédriques par hectare (CIP/ha) et la parcelle 2 une double application de $2,7 \times 10^{11}$ CIP/ha. Les traitements dosés à 9,4 L/ha ont eu lieu à 5 jours d'intervalle, lorsque les insectes en étaient à leur premier ou deuxième stade de développement.

L'arrosage des blocs a été très uniforme et a déclenché une épizootie virale dans les deux parcelles, les plus forts taux d'infection des larves étant de 46,7 et de 66,5% dans les parcelles 1 et 2 respectivement. Les taux d'infection les plus élevés par le virus naturel de la polyédrose nucléaire enregistrés dans les parcelles-témoins 1 et 2 étaient respectivement de 17,6 et de 27,9%. Dans les parcelles traitées, la densité des masses d'oeufs, de 1540/ha qu'elle était avant le traitement, est passée à 10/ha dans la parcelle 1 après le traitement et de 3240 à 1560/ha dans la parcelle 2. Les populations des parcelles-témoins ont également décliné de façon spectaculaire. Les résultats de ces essais montrent que le Disparvirus est un agent de lutte contre la spongieuse potentiellement efficace.

INTRODUCTION

Naturally occurring virus epizootics cause severe mortality in late instar larvae of gypsy moth, *Lymantria dispar* (L.), and are an important factor in terminating outbreaks (Podgwaite and Campbell 1971). The virus responsible, a nuclear polyhedrosis virus (NPV), is highly species specific. A strain of this NPV called Gypchek® was produced by the U.S. Forest Service and registered by the U.S. Environmental Protection Agency (EPA) in 1978. After extensive field evaluations in the USA, a double application with dosages ranging from 2.5×10^{11} to 1.25×10^{12} polyhedral inclusion bodies (PIBs)/ha in 18.8 L/ha was recommended against early instar larvae to obtain acceptable foliage protection and population reduction (Lewis et al. 1979).

The situation is similar in Canada, with naturally occurring virus epizootics developing after trees are severely defoliated. To evaluate NPV as an acceptable biological control agent for gypsy moth in Canada, field trials have been conducted. In 1982, 63 ha in the Kaladar area of Tweed District, Ontario were aerially sprayed with the lowest recommended dosage of Gypchek, obtained from the U.S. Forest Service. The virus reduced the insect population, protected foliage and gave results comparable to those obtained with the chemical insecticide Sevin® and the microbial insecticide *Bacillus thuringiensis, (B.t.)* (Meating et al. 1983). This report presents the results of aerial applications of Disparvirus, a Canadian NPV product. The lowest dosage recommended by the U.S. Forest Service and 10-fold this dosage were applied to populations of gypsy moth.

MATERIALS AND METHODS

The Virus

Disparvirus used in these trials was propagated in gypsy moth larvae reared in quarantine facilities at the Forest Pest Management Institute (FPMI) in 1985. Gypsy moth egg masses were supplied by the USDA Methods Development Centre, Otis Air Base, Massachusetts. About 500 larvae were initially infected with Gypchek to provide inoculum to propagate further quantities of this strain of NPV. Virus-infected larvae were harvested, frozen, freeze-dried and ground to a fine powder.

The Study Area

Two treatment plots were located in Olden and Kennebec Townships in southern Ontario. Plot 1, in Kennebec Twp., was a 10 ha site adjacent to a Ministry of Transport garage on Hwy. 7, 30 km west of Sharbot Lake. It consisted of mature red oak *(Quercus rubra* L.) and white oak *(Q. alba* L.) as well as sugar maple *(Acer saccharum* Marsh.), white ash *(Fraxinus americana* L.), and several species of softwood. Sampling conducted by Forest Insect and Disease Survey (FIDS) technicians revealed that there were 1540 gypsy moth egg masses/ha. Plot 2, in Olden Twp., was an 87.5 ha site at the Ontario Ministry of Natural Resources (OMNR) White Lake Fish Hatchery on Hwy. 7, 10 km west of Sharbot Lake. Stand composition was similar to plot 1 and egg mass density was 3420/ha. Two check areas were selected, one in Kennebec Twp. (check plot 1) and the other in Clarendon Twp. (check plot 2), with stand characteristics and gypsy moth populations as similar as possible to the treated blocks. These plots were far removed from the treated areas and there was no risk of accidental contamination due to drift from the spray application.

Larval Development

Before each spray application, 100 larvae were randomly collected from branch samples taken from each of the two treated plots. Instar was determined by head capsule measurement.

Mixing Procedures and Spray Application

Lyophilized NPV-infected insects were rehydrated and suspended in water using a Kalish® turbo homogenizer and then filtered through a 20 mesh sieve. An aqueous tank mix containing 25% molasses and 60 g/L Orzan LS, a lignosulfonate used as a sunscreen, was applied. Plots 1 and 2 were to receive double applications of 2.5 x 10^{12} PIBs/ha and 2.5 x 10^{11} PIBs/ha respectively. A Jet Ranger helicopter equipped with 4 Micronair® AU 4000 units, calibrated to deliver 9.4 L/ha, was used for both applications. Meteorological conditions, including relative humidity (R.H.) at the time of spraying were monitored at plot 2.

Deposit Sampling

The spray deposit was collected on Kromekote® cards mounted on aluminum backings and placed at 15 m intervals across each plot at right angles to the flight lines. The cards were collected approximately 30 min after spraying. An Artek 810 Image Analysis System was used to determine droplet density; 10 fields per card were examined, the total number of droplets was counted and the mean number of droplets/cm^2 was calculated.

Assessment of Levels of Virus Infection

To determine virus efficacy, 30 oak trees (red and white oak) were selected and tagged in each treatment and check plot. Burlap traps were tied to the trunks of these trees about 1.2 m from the ground. Pre-spray and weekly post-spray samples of larvae were collected from each tree commencing at 12 days post-spray. Insect samples were obtained by clipping branches at mid-crown with pole pruners and removing approximately 10 larvae from the foliage samples, until most of the larvae reached fourth instar. Late instar larvae, which spend limited time on the leaves, were collected from burlap traps and tree trunks. All larvae were squashed, smeared on glass slides and transported to FPMI. Smears were stained with Buffalo Black 12B and examined for the presence of PIBs at 1200X magnification.

Adult Emergence

Pupae were collected from burlap traps on the sample trees in all plots to determine if the virus application had any effect on adult emergence. They were reared individually and dead pupae were examined for NPV, other diseases, and parasites.

Gypsy Moth Egg Mass Counts

To assess gypsy moth population levels, five 0.01 ha sub-plots (10 X 10m) were established in each treatment area, and egg masses observed were used to estimate the density. Three sub-plots were located in untreated check areas. Differences in the mean number of egg masses laid in 1985 compared to those laid in 1986 provided an indication of the efficacy of the spray application.

Defoliation Estimates

Defoliation surveys were conducted by FIDS staff in the same sub-plots used for the egg mass counts. One 45-cm branch was cut from each of 10 trees in or adjacent to each sub-plot and defoliation was estimated by recording whether individual leaves were intact, totally eaten or partially eaten. A mean was calculated for each branch sample.

RESULTS

Spray Application

The first application of NPV started at 0550h, May 18 and took 105 min to complete with two loads. The R.H. was 82%. Larvae

were predominantly in the first instar in plot 1 and in the second instar in plot 2 (Table 1). The second application began at 0530h on May 22 and took 95 min to complete with two loads. The R.H. was 87%. Larvae in both plots were predominantly in the second instar (Table 1). After the spray application, samples from the spray tank of the helicopter were checked to verify the PIBs concentration. Results indicated that 2.2×10^{12} PIBs/ha and 2.7×10^{11} PIBs/ha were applied to plots 1 and 2 respectively at each application instead of 2.5×10^{12} PIBs/ha and 2.5×10^{11} PIBs/ha as originally planned.

The OMNR conducted a large operational spray with *B.t.* in the same general area and due to a misunderstanding check plot 2 was treated with *B.t.* 12 days after the second virus spray. Fortunately a crew taking larval samples was working in check plot 2 at the time of application and noticed the error.

Droplet Assessment

The droplet density on the Kromekote cards collected from plot 1 was 16.5 and 25.0 droplets/cm^2 for the two applications and 31.9 and 50.2 droplets/cm^2 in plot 2.

Table 1. Gypsy moth larval development at time of virus application

	First application				Second application			
		Instar				Instar		
Area	No. examined	I	II	III	No. examined	I	II	III
Plot 1	176	64%	35%	1%	254	34%	62%	4%
Plot 2	770	41%	58%	1%	264	28%	67%	5%

Levels of Virus Infection

Pre-spray sampling indicated that both the treated and check plots had low levels of naturally occurring NPV in the gypsy moth population (Table 2). At 12 days post-spray, plots 1 and 2 had 36.5% and 39.9% of larvae respectively, infected with virus compared to the check areas which had 12.6% and 14.8% of larvae infected (Table 2). Levels of virus infection in plot 1 increased to 46.7% after 19 days, the highest observed for this plot. Infection rates decreased thereafter, and at 42 days post-spray 25.2% of the remaining population was infected with NPV. In plot 2, 57.2% of the larvae were infected with NPV at 19 days post-spray; this level decreased at 26 days post-spray, then gradually increased to the highest level observed in this plot, 66.5%, at 42 days post-spray. A naturally occurring epizootic developed in both check plots, although faster in check plot 2. The last collection of larvae from the check plots revealed 17.6% and 27.9% of larvae infected with NPV in check plots 1 and 2 respectively (Fig. 1).

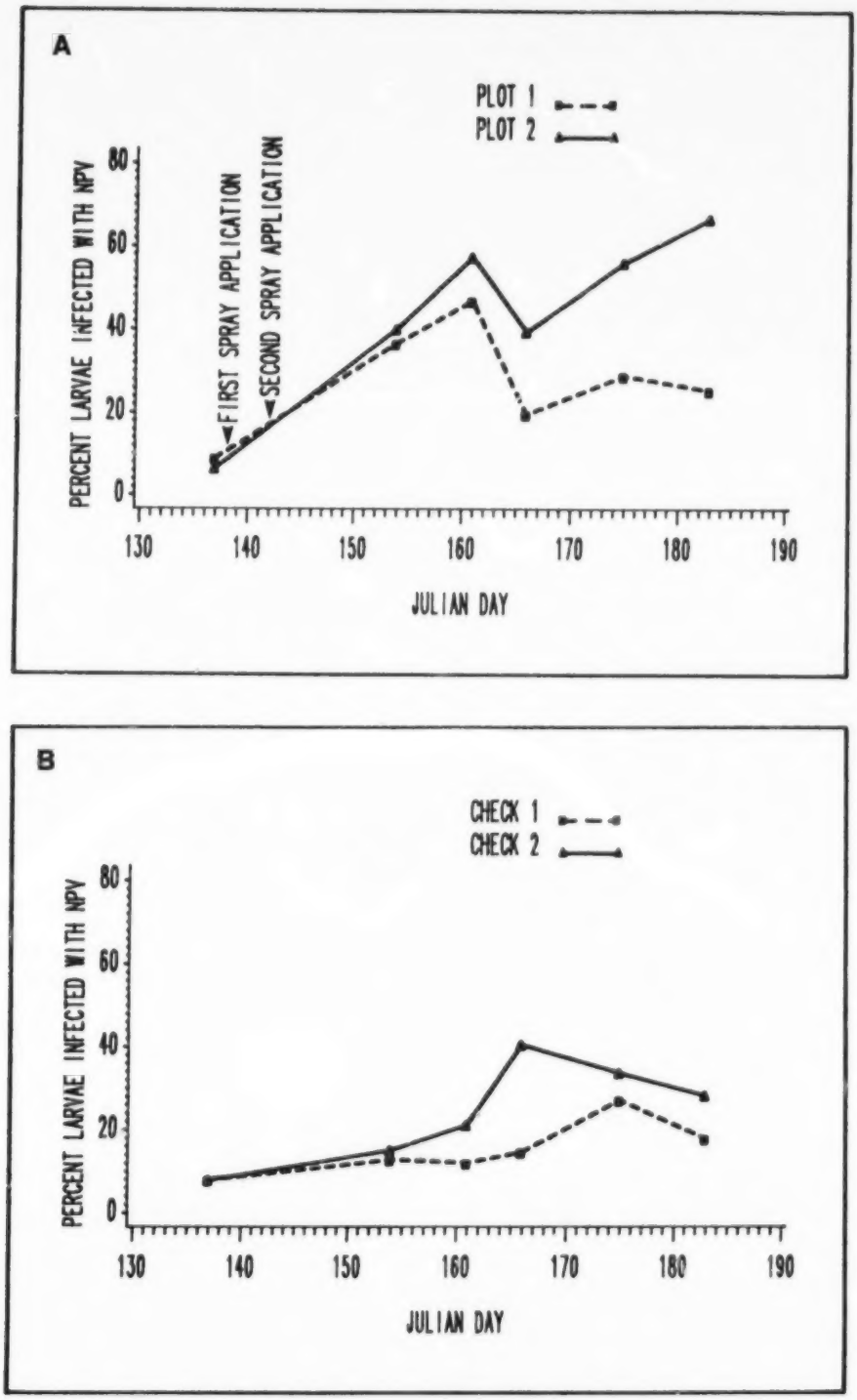

Fig. 1. Development of viral epizootics in treated plots (A), and in untreated check plots (B).

Table 2. Incidence (%) of NPV in gypsy moth larvae collected from NPV-treated plots and check plots in 1986. The number of larvae in each sample is shown in parentheses

Plot	Pre-spray	Days post-spray				
		12	19	26	33	42
1	8.8 (327)	36.5 (340)	46.7 (274)	19.5 (128)	28.8 (111)	25.2 (167)
2	6.3 (333)	39.9 (328)	57.2 (311)	39.2 (291)	55.9 (286)	66.5 (266)
Check 1	7.7 (324)	12.6 (309)	11.8 (315)	14.4 (305)	26.9 (293)	17.6 (302)
Check 2*	7.6 (290)	14.8 (329)	20.9 (316)	40.2 (286)	33.4 (284)	27.9 (391)

* aerially sprayed with *Bacillus thuringiensis*.

Adult Emergence

Examination of pupae collected and reared to adult emergence indicated no difference in mortality from virus between treated and untreated plots (Table 3). Fewer females than males emerged from pupae collected in 3 of the 4 plots and the incidence of parasitism was higher in check plots than in treated plots.

Defoliation Estimates

Defoliation estimates from the treated and check plots indicated that the application of Disparvirus gave no demonstrable foliage protection. Defoliation was light to moderate in all plots. Red oak trees in plots 1 and 2 suffered 39% and 22% defoliation respectively. There was 18% defoliation in check plot 1 and 40% in check plot 2 (Table 4).

Table 3. Adult emergence from gypsy moth pupae collected in treated and check plots in 1986

Plot	Total examined	% successful adult emergence	% adult females	% adult males	% emerged adult parasites	% dead with NPV	% dead from unknown
1	84	78.6	26.2	52.4	9.5	9.5	2.4
2	306	69.3	36.3	33.0	14.7	9.8	6.2
Check 1	455	63.5	21.1	42.4	25.1	8.6	2.8
Check 2	647	45.3	21.0	24.3	35.4	6.8	12.4

Table 4. Average defoliation on red oak (rO) in treated and check plots

Area	No. of sub-plots	% Mean defoliation rO
Plot 1	5	22
Plot 2	5	39
Check 1	1	18
Check 2	2	40

Gypsy Moth Egg Mass Counts

Egg mass counts revealed that the gypsy moth population collapsed in both check areas with 95% and 80% reductions in plots 1 and 2 respectively (Table 5). In the treated plots, egg mass density fell from a pre-spray count of 1540/ha to a post-spray count of 10/ha in plot 1, and from 3240/ha to 1560/ha in plot 2. This represented population declines of 99% and 52% respectively.

Table 5. Changes in egg mass densities in treated and check areas

Area	No. of sub-plots	Mean number of eggs masses per ha 1985	1986	% Decline
Plot 1	5	15400	10	99
Plot 2	5	32400	15600	52
Check 1	1	107000	5000	95
Check 2	2	46500	9500	80

DISCUSSION

A dosage of 2.2 X 10^{12} PIBs/ha, 10-fold the lowest recommended dosage and 2-fold the highest recommended dosage, annihilated the gypsy moth population in plot 1. In plot 2, treatment with 2.7 X 10^{11} PIBs/ha created an epizootic which caused considerable mortality in the population. However, it only caused a 52% reduction in egg mass density and did little to decrease defoliation because of the very high pre-spray density of larvae. Foliage protection was difficult to assess. First, there were considerable differences in population densities between treated and check plots and second, the slow-acting nature of NPV infection predisposes trees to some defoliation, since larvae continue to feed after becoming infected.

Graphs of larval infection over time show that application of Disparvirus created a more intense epizootic in earlier instar larvae than was observed in check plots (Fig. 1). In the treated plots, initial peaks of infection occurred approximately 18 days after the second application of NPV. Lower levels of infection, observed in both plots at the third post-spray sample, represent a period of abatement in the epizootic as insects initially infected by Disparvirus disappear. Infection levels gradually increase as the remaining larvae are infected by horizontal transmission of the NPV. Differences in the levels of infection are the result of differences in population densities between plots 1 and 2. In contrast, the peak of the naturally occurring epizootics in the check plots did not appear until 32 days after the second application (check plot 1), and was at a lower level than in the treated plots. A premature peak observed for check plot 2 may have been the result of the *B.t.* treatment which released NPV inoculum from infected larvae earlier than normal.

If a sample had been taken at one week post-spray, it may have revealed a peak of larval infection in plot 1. Persons collecting larvae noticed a major difference in population density in plot 1 between the pre-spray sample and the 12 day post-spray sample, and it is probable that the spray deposit caused a major population reduction leaving comparatively few larvae to succumb

to horizontally transmitted NPV. This observation indicates that it may be possible to assess the impact of applications of control agents on gypsy moth by counting early-instar larvae on branch samples.

The application of NPV had no effect on pupal mortality (Table 3). The virus treatments reduced the emergence of adult parasites. Presumably, the virus killed parasitized larvae and the immature larval parasites also perished. Virus diseases affect the sex ratios of several lepidopterous and hymenopterous species because females are longer in the larval stage than males and have a greater chance of succumbing to disease (Cunningham 1982). This is so for several species of lymantriids, including the gypsy moth, where the female has one more instar than the male.

Results from these trials indicate that Disparvirus is a potentially effective control agent for gypsy moth. The dosage of NPV and gypsy moth population density are two important factors to consider. The high dosage, which was successful in this trial, is uneconomical to recommend for operational use now. Consequently, future work will be directed at generating efficacy data based on dosages greater than 2.7×10^{11} PIBs/ha and less than 2.2×10^{12} PIBs/ha.

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